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       07jan02 12:44:50 User242957 Session D366.1
            $0.00    0.203 DialUnits FileHomeBase
     $0.00  Estimated cost FileHomeBase
     $0.00  Estimated cost this search
     $0.00  Estimated total session cost   0.203 DialUnits

File 410:Chronolog(R)  1981-2001/Nov
       (c) 2001 The Dialog Corporation

      Set  Items  Description
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? set hi ;set hi

HILIGHT set on as ''
HILIGHT set on as ''
? b 155, 5

       07jan02 12:44:55 User242957 Session D366.2
            $0.00    0.065 DialUnits File410
     $0.00  Estimated cost File410
     $0.00  Estimated cost this search
     $0.00  Estimated total session cost   0.268 DialUnits

SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R)  1966-2002/JAN W2
*File 155: Updates include In Process records only. Updating of
Completed records is expected to resume in January. See Help News155.
  File   5:Biosis Previews(R)  1969-2001/Dec W5
         (c) 2001 BIOSIS

      Set  Items  Description
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? e au=breaker ronald


Ref    Items  Index-term
E1         1  AU=BREAKER LAURENCE C
E2         5  AU=BREAKER R R
E3         0 *AU=BREAKER RONALD
E4        35  AU=BREAKER RONALD R
E5        40  AU=BREAKER RR
E6         1  AU=BREAKEVELT C R
E7         4  AU=BREAKEY A S
E8         4  AU=BREAKEY AS
E9         1  AU=BREAKEY B
E10        1  AU=BREAKEY BM
E11        1  AU=BREAKEY C
E12        1  AU=BREAKEY D

          Enter P or PAGE for more
? s 45-e5

      S1       0  45-E5
? s e4-e5

              35  AU=BREAKER RONALD R
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            40  AU=BREAKER RR
      S2    75  E4-B
? s s2 and ribozym?

            75  S2
          5506  RIBOZYM?
      S3    44  S2 AND RIBOZYM?
? rd

...completed examining records
      S4    26  RD (unique items)
? t s4/3,ab/all


 4/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11550902   21180123   PMID: 11283591
  Immobilized RNA switches for the analysis of complex chemical and
biological mixtures.
  Seetharaman S; Zivarts M; Sudarsan N; Breaker RR
  Department of Molecular, Cellular and Developmental Biology, Yale
University, New Haven, CT 06520-8103, USA.
  Nature biotechnology (United States) Apr 2001, 19 (4) p336-41,
ISSN 1087-0156 Journal Code: CQ3
  Comment in Nat Biotechnol. 2001 Apr;19(4) 313-4; Comment in/MedlineID
21180111
  Languages: ENGLISH
  Document type: Journal Article
  Record type: Completed
  A prototype biosensor array has been assembled from engineered RNA
molecular switches that undergo ribozyme-mediated self-cleavage when
triggered by specific effectors. Each type of switch is prepared with a
5'-thiotriphosphate moiety that permits immobilization on gold to form
individually addressable pixels. The ribozymes comprising each pixel
become active only when presented with their corresponding effector, such
that each type of switch serves as a specific analyte sensor. An addressed
array created with seven different RNA switches was used to report the
status of targets in complex mixtures containing metal ion, enzyme
cofactor, metabolite, and drug analytes. The RNA switch array also was used
to determine the phenotypes of Escherichia coli strains for adenylate
cyclase function by detecting naturally produced 3',5'- cyclic adenosine
monophosphate (cAMP) in bacterial culture media.


 4/3,AB/2     (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11256939   21241686   PMID: 11345431
  Generating new ligand-binding RNAs by affinity maturation and
disintegration of allosteric ribozymes.
  Soukup GA; DeRose EC; Koizumi M; Breaker RR
  Department of Molecular, Cellular and Developmental Biology, Yale
University, New Haven, Connecticut 06520-8103, USA.
  RNA (United States) Apr 2001, 7 (4) p524-36, ISSN 1355-8382
Journal Code: CHB
  Contract/Grant No.: GM559343, GM, NIGMS
  Languages: ENGLISH
  Document type: Journal Article
  Record type: In Process
  Allosteric ribozymes are engineered RNAs that operate as molecular
switches whose rates of catalytic activity are modulated by the binding of
specific effector molecules. New RNA molecular switches can be created by
using "allosteric selection," a molecular engineering process that combines
modular rational design and in vitro evolution strategies. In this report,
```

we describe the characterization of 3',5'-cyclic nucleotide monophosphate (cNMP)-dependent hammerhead **ribozymes** that were isolated using allosteric selection (Koizumi et al., Nat Struct Biol, 1999, 6:1062-1071). Artificial phylogeny data generated by random mutagenesis and reselection of existing cGMP-, cCMP-, and cAMP-dependent **ribozymes** indicate that each is comprised of distinct effector-binding and catalytic domains. In addition, patterns of nucleotide covariation and direct mutational analysis both support distinct secondary-structure organizations for the effector-binding domains. Guided by these structural models, we were able to disintegrate each allosteric **ribozyme** into separate ligand-binding and catalytic modules. Examinations of the independent effector-binding domains reveal that each retains its corresponding cNMP-binding function. These results validate the use of allosteric selection and modular engineering as a means of simultaneously generating new nucleic acid structures that selectively bind ligands. Furthermore, we demonstrate that the binding affinity of an allosteric **ribozyme** can be improved through random mutagenesis and allosteric selection under conditions that favor tighter binding. This "affinity maturation" effect is expected to be a valuable attribute of allosteric selection as future endeavors seek to apply engineered allosteric **ribozymes** as biosensor components and as controllable genetic switches.

4/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11243658 21169368 PMID: 11266567
Cooperative binding of effectors by an allosteric **ribozyme**.
Jose AM; Soukup GA; **Breaker RR**
Department of Molecular, Cellular and Developmental Biology, KBT 452, Yale University, PO Box 208103, New Haven, CT 06520-8103, USA.
Nucleic acids research (England) Apr 1 2001, 29 (7) p1631-7, ISSN 1362-4962 Journal Code: DF1
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
An allosteric **ribozyme** that requires two different effectors to induce catalysis was created using modular rational design. This **ribozyme** construct comprises five conjoined RNA modules that operate in concert as an obligate FMN- and theophylline-dependent molecular switch. When both effectors are present, this 'binary' RNA switch self-cleaves with a rate enhancement of approximately 300-fold over the rate observed in the absence of effectors. Kinetic and structural studies implicate a switching mechanism wherein FMN binding induces formation of the active **ribozyme** conformation. However, the binding site for FMN is rendered inactive unless theophylline first binds to its corresponding site and reorganizes the RNA structure. This example of cooperative binding between allosteric effectors reveals a level of structural and functional complexity for RNA that is similar to that observed with allosteric proteins.

4/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11040129 21060020 PMID: 10542100
Allosteric selection of **ribozymes** that respond to the second messengers cGMP and cAMP.
Koizumi M; Soukup GA; Kerr JN; **Breaker RR**
Department of Molecular, Cellular and Developmental Biology, Yale University, P.O. Box 208103, New Haven, Connecticut 06520-8103, USA.
Nature structural biology (United States) Nov 1999, 6 (11) p1062-71, ISSN 1072-8368 Journal Code: B98
Languages: ENGLISH
Document type: Journal Article

Record type: In Process
RNA transcripts containing the hammerhead **ribozyme** have been engineered to self-destruct in the presence of specific nucleoside 3',5'-cyclic monophosphate compounds. These RNA molecular switches were created by a new combinatorial strategy termed 'allosteric selection,' which favors the emergence of **ribozymes** that rapidly self-cleave only when incubated with their corresponding effector compounds. Representative RNAs exhibit 5,000-fold activation upon cGMP or cAMP addition, display precise molecular recognition characteristics, and operate with catalytic rates that match those exhibited by unaltered **ribozymes**. These findings demonstrate that a vast number of ligand-responsive **ribozymes** with dynamic structural characteristics can be generated in a massively parallel fashion. Moreover, optimized allosteric **ribozymes** could serve as highly selective sensors of chemical agents or as unique genetic control elements for the programmed destruction of cellular RNAs.

4/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10635290 20283903 PMID: 10823936
Structural diversity of self-cleaving **ribozymes**.
Tang J; **Breaker RR**
Department of Molecular, Cellular and Developmental Biology, Yale University, P.O. Box 208103, New Haven, CT 06520-8103, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) May 23 2000, 97 (11) p5784-9, ISSN 0027-8424 Journal Code: PV3
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
In vitro selection was used to isolate Mg(2+)-dependent self-cleaving **ribozymes** from random sequence. Characterization of representative clones revealed the emergence of at least 12 classes of **ribozymes** that adopt distinct secondary structure motifs. Only one class corresponds to a previously known structural motif, that of the naturally occurring hammerhead **ribozyme**. Each **ribozyme** promotes self-cleavage via an internal phosphoester transfer reaction involving the adjacent 2'-hydroxyl group with a chemical rate enhancement of between 10(3)- and 10(6)-fold greater than the corresponding uncatalyzed rate. These findings indicate that RNA can form a multitude of secondary and tertiary structures that promote cleavage by internal phosphoester transfer. Upon further in vitro selection, a class I **ribozyme** that adopts an "X motif" structure dominates over all other **ribozymes** in the population. Thus, self-cleaving RNAs isolated by in vitro selection from random-sequence populations can rival the catalytic efficiency of natural **ribozymes**.

4/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10628991 20241398 PMID: 10780486
Allosteric **ribozymes** sensitive to the second messengers cAMP and cGMP.
Koizumi M; Kerr JN; Soukup GA; **Breaker RR**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA.
Nucleic acids symposium series (ENGLAND) 1999, (42) p275-6, ISSN 0261-3166 Journal Code: O8N
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
We have engineered allosteric **ribozymes** by combining modular rational design with combinatorial strategies. This new procedure was used

to create allosteric **ribozymes** that are activated by specific nucleoside 3',5'-cyclic monophosphates (cNMPs). A random-sequence domain was attached to stem II of hammerhead **ribozymes** via a communication module that serves as an interface between **ribozyme** and the effector binding site. Subjecting this initial random pool to in vitro selection methods produced populations that respond, or cleave, only in the presence of specific effector molecules. From generation 18, 20 and 23, cGMP, cCMP and cAMP-specific responsive **ribozymes**, respectively, were isolated and characterized. These methods show great promise for engineering allosteric **ribozymes** and for creating new ligand-specific aptamers.

4/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10605427 20251042 PMID: 10788325
Altering molecular recognition of RNA aptamers by allosteric selection.
Soukup GA; Emilsson GA; **Breaker RR**
Department of Molecular Cellular and Developmental Biology, Yale University, New Haven, CT 06520-8103, USA.
Journal of molecular biology (ENGLAND) May 12 2000, 298 (4) p623-32, ISSN 0022-2836 Journal Code: J6V
Contract/Grant No.: GM559343, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
In a continuing effort to explore structural and functional dynamics in RNA catalysis, we have created a series of allosteric hammerhead **ribozymes** that are activated by theophylline. Representative **ribozymes** exhibit greater than 3000-fold activation upon effector-binding and cleave with maximum rate constants that are equivalent to the unmodified hammerhead **ribozyme**. In addition, we have evolved a variant allosteric **ribozyme** that exhibits an effector specificity change from theophylline to 3-methylxanthine. Molecular discrimination between the two effectors appears to be mediated by subtle conformational differences that originate from displacement of the phosphodiester backbone near the effector binding pocket. These findings reveal the importance of abstruse aspects of molecular recognition by nucleic acids that are likely to be unapproachable by current methods of rational design. Copyright 2000 Academic Press.

4/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10422985 20025500 PMID: 10557159
Nucleic acid molecular switches.
Soukup GA; **Breaker RR**
Department of Molecular, Cellular and Developmental Biology, Yale University, PO Box 208103, New Haven, CT 06520-8103, USA. garrett.soukup@yale.edu
Trends in biotechnology (ENGLAND) Dec 1999, 17 (12) p469-76, ISSN 0167-7799 Journal Code: ALJ
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed
Natural and artificial **ribozymes** can catalyse a diverse range of chemical reactions. Through recent efforts in enzyme engineering, it has become possible to tailor the activity of **ribozymes** to respond allosterically to specific effector compounds. These allosteric **ribozymes** function as effector-dependent molecular switches that could find application as novel genetic-control elements, biosensor components or precision switches for use in nanotechnology.

4/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDL[redacted](R)

10392264 20037793 PMID: 10573122
Relationship between internucleotide linkage geometry and the stability of RNA.
Soukup GA; **Breaker RR**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520-8103, USA.
RNA (UNITED STATES) Oct 1999, 5 (10) p1308-25, ISSN 1355-8382 Journal Code: CHB
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The inherent chemical instability of RNA under physiological conditions is primarily due to the spontaneous cleavage of phosphodiester linkages via intramolecular transesterification reactions. Although the protonation state of the nucleophilic 2'-hydroxyl group is a critical determinant of the rate of RNA cleavage, the precise geometry of the chemical groups that comprise each internucleotide linkage also has a significant impact on cleavage activity. Specifically, transesterification is expected to be proportional to the relative in-line character of the linkage. We have examined the rates of spontaneous cleavage of various RNAs for which the secondary and tertiary structures have previously been modeled using either NMR or X-ray crystallographic data. Rate constants determined for the spontaneous cleavage of different RNA linkages vary by almost 10,000-fold, most likely reflecting the contribution that secondary and tertiary structures make towards the overall chemical stability of RNA. Moreover, a correlation is observed between RNA cleavage rate and the relative in-line fitness of each internucleotide linkage. One linkage located within an ATP-binding RNA aptamer is predicted to adopt most closely the ideal conformation for in-line attack. This linkage has a rate constant for transesterification that is approximately 12-fold greater than is observed for an unconstrained linkage and was found to be the most labile among a total of 136 different sites examined. The implications of this relationship for the chemical stability of RNA and for the mechanisms of nucleases and **ribozymes** are discussed.

4/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10345594 99354412 PMID: 10425680
Design of allosteric hammerhead **ribozymes** activated by ligand-induced structure stabilization.
Soukup GA; **Breaker RR**
Department of Molecular, Yale University, New Haven, Connecticut 06520-8103, USA.
Structure with Folding & design (ENGLAND) Jul 15 1999, 7 (7) p783-91, ISSN 0969-2126 Journal Code: DEB
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
BACKGROUND: **Ribozymes** can function as allosteric enzymes that undergo a conformational change upon ligand binding to a site other than the active site. Although allosteric **ribozymes** are not known to exist in nature, nucleic acids appear to be well suited to display such advanced forms of kinetic control. Current research explores the mechanisms of allosteric **ribozymes** as well as the strategies and methods that can be used to create new controllable enzymes. RESULTS: In this study, we exploit the modular nature of certain functional RNAs to engineer allosteric **ribozymes** that are activated by flavin mononucleotide (FMN) or theophylline. By joining an FMN- or theophylline-binding domain to a hammerhead **ribozyme** by different stem II elements, we have identified a minimal connective bridge comprised of a G.U wobble pair that

[redacted]

is responsive to ligand binding. Binding of FMN or theophylline to its allosteric site induces a conformational change in the RNA that stabilizes the wobble pair and ultimately favors the active form of the catalytic core. These ligand-sensitive **ribozymes** exhibit rate enhancements of more than 100-fold in the presence of FMN and of approximately 40-fold in the presence of theophylline. CONCLUSIONS: An adaptive strategy for modular rational design has proven to be an effective approach to the engineering of novel allosteric **ribozymes** . This strategy was used to create allosteric **ribozymes** that function by a mechanism involving ligand-induced structure stabilization. Conceivably, similar engineering strategies and allosteric mechanisms could be used to create a variety of novel allosteric **ribozymes** that function with other effector molecules.

4/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10112907 99199227 PMID: 10097080
Engineering precision RNA molecular switches.
Soukup GA; **Breaker RR**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520-8103, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 30 1999, 96 (7) p3584-9, ISSN 0027-8424 Journal Code: PV3
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Ligand-specific molecular switches composed of RNA were created by coupling preexisting catalytic and receptor domains via structural bridges. Binding of ligand to the receptor triggers a conformational change within the bridge, and this structural reorganization dictates the activity of the adjoining **ribozyme**. The modular nature of these tripartite constructs makes possible the rapid construction of precision RNA molecular switches that trigger only in the presence of their corresponding ligand. By using similar enzyme engineering strategies, new RNA switches can be made to operate as designer molecular sensors or as a new class of genetic control elements.

4/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09924919 98391746 PMID: 9722642
Mechanism for allosteric inhibition of an ATP-sensitive **ribozyme**.
Tang J; **Breaker RR**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520-8103, USA.
Nucleic acids research (ENGLAND) Sep 15 1998, 26 (18) p4214-21, ISSN 0305-1048 Journal Code: O8L
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
We report the structural basis for the modulation of an ATP-sensitive **ribozyme** that was engineered by modular rational design. This allosteric **ribozyme** is composed of two independently functioning domains, one a receptor for ATP and the other a self-cleaving **ribozyme** . When fused in the appropriate fashion, the conjoined aptamer-**ribozyme** construct functions as an allosteric **ribozyme** that is inhibited in the presence of ATP. The aptamer domain remains conformationally heterogeneous in the absence of ATP, but folds into a distinct structure upon ligand binding. This ATP-induced conformational change causes a reduction in catalytic activity of the adjacent **ribozyme** domain due to steric interference between the aptamer and

[redacted]

ribozyme tertiary structures. This mechanism for structural and functional modulation of nucleic acids is one of several possible mechanisms by which the function of **ribozymes** could be specifically controlled by small effector molecules.

4/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09848020 98374603 PMID: 9667831
DNA aptamers and DNA enzymes.
Breaker RR
Department of Biology, KBT 452, Yale University, PO Box 208103, New Haven, CT 06520, USA. ronald.breaker@yale.edu
Current opinion in chemical biology (ENGLAND) Jun 1997, 1 (1) p26-31, ISSN 1367-5931 Journal Code: C4U
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed
Investigators using combinatorial methods are revealing the surprising structural and functional abilities of DNA. A consequence of DNA's structure-forming potential is its ability to form highly specific receptors and ligands, and even its ability to catalyze chemical reactions. Unlike the classical images of double-stranded DNA, these DNA structures have many of the higher-ordered structural features that are found with **ribozymes** and other folded RNAs. Recent research is beginning to indicate that these new DNA structures are not rare exceptions, and that DNA, despite the absence of 2' hydroxyl groups, could rival RNA in its ability to form intricate structures and in its ability to function as an enzyme.

4/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09771591 98263302 PMID: 9600911
An amino acid as a cofactor for a catalytic polynucleotide.
Roth A; **Breaker RR**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520-8103, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) May 26 1998, 95 (11) p6027-31, ISSN 0027-8424 Journal Code: PV3
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Natural **ribozymes** require metal ion cofactors that aid both in structural folding and in chemical catalysis. In contrast, many protein enzymes produce dramatic rate enhancements using only the chemical groups that are supplied by their constituent amino acids. This fact is widely viewed as the most important feature that makes protein a superior polymer for the construction of biological catalysts. Herein we report the in vitro selection of a catalytic DNA that uses histidine as an active component for an RNA cleavage reaction. An optimized deoxyribozyme from this selection requires L-histidine or a closely related analog to catalyze RNA phosphoester cleavage, producing a rate enhancement of approximately 1-million-fold over the rate of substrate cleavage in the absence of enzyme. Kinetic analysis indicates that a DNA-histidine complex may perform a reaction that is analogous to the first step of the proposed catalytic mechanism of RNase A, in which the imidazole group of histidine serves as a general base catalyst. Similarly, **ribozymes** of the "RNA world" may have used amino acids and other small organic cofactors to expand their otherwise limited catalytic potential.

4/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDL[redacted](R)

09545293 97400133 PMID: 9257650
Examination of the catalytic fitness of the hammerhead **ribozyme** by in vitro selection.
Tang J; **Breaker RR**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520-8103, USA.
RNA (UNITED STATES) Aug 1997, 3 (8) p914-25, ISSN 1355-8382 Journal Code: CHB
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
We have designed a self-cleaving **ribozyme** construct that is rendered inactive during preparative in vitro transcription by allosteric interactions with ATP. This allosteric **ribozyme** was constructed by joining a hammerhead domain to an ATP-binding RNA aptamer, thereby creating a **ribozyme** whose catalytic rate can be controlled by ATP. Upon purification by PAGE, the engineered **ribozyme** undergoes rapid self-cleavage when incubated in the absence of ATP. This strategy of "allosteric delay" was used to prepare intact hammerhead **ribozymes** that would otherwise self-destruct during transcription. Using a similar strategy, we have prepared a combinatorial pool of RNA in order to assess the catalytic fitness of **ribozymes** that carry the natural consensus sequence for the hammerhead. Using in vitro selection, this comprehensive RNA pool was screened for sequence variants of the hammerhead **ribozyme** that also display catalytic activity. We find that sequences that comprise the core of naturally occurring hammerhead dominate the population of selected RNAs, indicating that the natural consensus sequence of this **ribozyme** is optimal for catalytic function.

4/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09390442 97370078 PMID: 9224568
Rational design of allosteric **ribozymes**.
Tang J; **Breaker RR**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, 06520-8103, USA.
Chemistry & biology (ENGLAND) Jun 1997, 4 (6) p453-9, ISSN 1074-5521 Journal Code: CNA
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
BACKGROUND: Efficient operation of cellular processes relies on the strict control that each cell exerts over its metabolic pathways. Some protein enzymes are subject to allosteric regulation, in which binding sites located apart from the enzyme's active site can specifically recognize effector molecules and alter the catalytic rate of the enzyme via conformational changes. Although RNA also performs chemical reactions, no **ribozymes** are known to operate as true allosteric enzymes in biological systems. It has recently been established that small-molecule receptors can readily be made of RNA, as demonstrated by the in vitro selection of various RNA aptamers that can specifically bind corresponding ligand molecules. We set out to examine whether the catalytic activity of an existing **ribozyme** could be brought under the control of an effector molecule by designing conjoined aptamer-**ribozyme** complexes. RESULTS: By joining an ATP-binding RNA to a self-cleaving **ribozyme**, we have created the first example of an allosteric **ribozyme** that has a catalytic rate that can be controlled by ATP. A 180-fold reduction in rate is observed upon addition of either adenosine or ATP, but no inhibition is detected in the presence of dATP or other nucleoside triphosphates. Mutations in the aptamer domain that are expected to

[redacted]

eliminate ATP binding or that increase the distance between aptamer and **ribozyme** domains result in a loss of ATP-specific allosteric control. Using a similar design approach, allosteric hammerhead **ribozymes** that are activated in the presence of ATP were created and another **ribozyme** that can be controlled by theophylline was created. CONCLUSIONS: The catalytic features of these conjoined aptamer-**ribozyme** constructs demonstrate that catalytic RNAs can also be subject to allosteric regulation-a key feature of certain protein enzymes. Moreover, by using simple rational design strategies, it is now possible to engineer new catalytic polynucleotides which have rates that can be tightly and specifically controlled by small effector molecules.

4/3,AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09371991 97278312 PMID: 9131619
DNA enzymes.
Breaker RR
Department of Biology, Yale University, New Haven, CT 06520-8103, USA. ronald.breaker@yale.edu
Nature biotechnology (UNITED STATES) May 1997, 15 (5) p427-31, ISSN 1087-0156 Journal Code: CQ3
Languages: ENGLISH
Document type: Journal Article; Review; Review, Academic
Record type: Completed
Biological catalysis is dominated by enzymes that are made of protein, but several distinct classes of catalytic RNAs are known to promote chemical transformations that are fundamental to cellular metabolism. Is biological catalysis limited only to these two biopolymers, or is DNA also capable of functioning as an enzyme in nature? To date, no DNA enzymes of natural origin have been found. However, an increasing number of catalytic DNAs, with characteristics that are similar to those of **ribozymes**, are being produced outside the confines of the cell. An assessment of the potential for structure formation by DNA leads to the conclusion that DNA might have considerable latent potential for enzymatic function.

4/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09124120 97153183 PMID: 9000012
In vitro selection of self-cleaving DNAs.
Carmi N; Shultz LA; **Breaker RR**
Department of Biology, Yale University, New Haven, CT 06520-8103, USA.
Chemistry & biology (ENGLAND) Dec 1996, 3 (12) p1039-46, ISSN 1074-5521 Journal Code: CNA
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
BACKGROUND: **Ribozymes** catalyze an important set of chemical transformations in metabolism, and 'engineered' **ribozymes** have been made that catalyze a variety of additional reactions. The possibility that catalytic DNAs or 'deoxyribozymes' can be made has only recently been addressed. Specifically, it is unclear whether the absence of the 2' hydroxyl renders DNA incapable of exhibiting efficient enzyme-like activity, making it impossible to discover natural or create artificial DNA biocatalysts. RESULTS: We report the isolation by in vitro selection of two distinct classes of self-cleaving DNAs from a pool of random-sequence oligonucleotides. Individual catalysts from 'class I' require both Cu2+ and ascorbate to mediate oxidative self-cleavage. Individual catalysts from class II use Cu2+ as the sole cofactor. Further optimization of a class II individual by in vitro selection yielded new catalytic DNAs that facilitate Cu2+-dependent self-cleavage with rate enhancements exceeding 1 000 000-fold relative to the uncatalyzed rate of DNA cleavage. CONCLUSIONS:

Despite the absence of 2' hydroxyls, single-stranded DNA can adopt structures that promote divalent-metal-dependent self-cleavage via an oxidative mechanism. These results suggest that an efficient DNA enzyme might be made to cleave DNA in a biological context.

4/3,AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08814884 98044642 PMID: 9383394
A DNA enzyme that cleaves RNA.
Breaker RR; Joyce GF
Department of Chemistry, Scripps Research Institute, La Jolla, CA 92037, USA.
Chemistry & biology (ENGLAND) Dec 1994, 1 (4) p223-9, ISSN 1074-5521 Journal Code: CNA
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
BACKGROUND: Several types of RNA enzymes (**ribozymes**) have been identified in biological systems and generated in the laboratory. Considering the variety of known RNA enzymes and the similarity of DNA and RNA, it is reasonable to imagine that DNA might be able to function as an enzyme as well. No such DNA enzyme has been found in nature, however. We set out to identify a metal-dependent DNA enzyme using in vitro selection methodology. RESULTS: Beginning with a population of 10(14) DNAs containing 50 random nucleotides, we carried out five successive rounds of selective amplification, enriching for individuals that best promote the Pb(2+)-dependent cleavage of a target ribonucleoside 3'-O-P bond embedded within an otherwise all-DNA sequence. By the fifth round, the population as a whole carried out this reaction at a rate of 0.2 min-1. Based on the sequence of 20 individuals isolated from this population, we designed a simplified version of the catalytic domain that operates in an intermolecular context with a turnover rate of 1 min-1. This rate is about 10(5)-fold increased compared to the uncatalyzed reaction. CONCLUSIONS: Using in vitro selection techniques, we obtained a DNA enzyme that catalyzes the Pb(2+)-dependent cleavage of an RNA phosphoester in a reaction that proceeds with rapid turnover. The catalytic rate compares favorably to that of known RNA enzymes. We expect that other examples of DNA enzymes will soon be forthcoming.

4/3,AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08580073 95371129 PMID: 7643406
Self-incorporation of coenzymes by **ribozymes**.
Breaker RR; Joyce GF
Department of Chemistry, Scripps Research Institute, La Jolla, CA 92037, USA.
Journal of molecular evolution (UNITED STATES) Jun 1995, 40 (6) p551-8, ISSN 0022-2844 Journal Code: J76
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
RNA molecules that are assembled from the four standard nucleotides contain a limited number of chemical functional groups, a characteristic that is generally thought to restrict the potential for catalysis by **ribozymes** . Although polypeptides carry a wider range of functional groups, many contemporary protein-based enzymes employ coenzymes to augment their capabilities. The coenzymes possess additional chemical moieties that can participate directly in catalysis and thereby enhance catalytic function. In this work, we demonstrate a mechanism by which **ribozymes** can supplement their limited repertoire of functional groups through RNA-catalyzed incorporation of various coenzymes and coenzyme analogues.

The group I **ribozyme** of Tetrahymena thermophila normally mediates a phosphoester transfer reaction that results in the covalent attachment of guanosine to the **ribozyme**. Here, a shortened version of the **ribozyme** is shown to catalyze the self-incorporation of coenzymes and coenzyme analogues, such as NAD+ and dephosphorylated CoA-SH. Similar **ribozyme** activities may have played an important role in the "RNA world," when RNA enzymes are thought to have maintained a complex metabolism in the absence of proteins and would have benefited from the inclusion of additional functional groups.

4/3,AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08391348 94331211 PMID: 7519862
Inventing and improving **ribozyme** function: rational design versus iterative selection methods.
Breaker RR; Joyce GF
Department of Chemistry, Scripps Research Institute, La Jolla, CA 92037.
Trends in biotechnology (ENGLAND) Jul 1994, 12 (7) p268-75, ISSN 0167-7799 Journal Code: ALJ
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed
Two major strategies for generating novel biological catalysts exist. One relies on our knowledge of biopolymer structure and function to aid in the 'rational design' of new enzymes. The other, often called 'irrational design', aims to generate new catalysts, in the absence of detailed physicochemical knowledge, by using selection methods to search a library of molecules for functional variants. Both strategies have been applied, with considerable success, to the remodeling of existing **ribozymes** and the development of **ribozymes** with novel catalytic function. The two strategies are by no means mutually exclusive, and are best applied in a complementary fashion to obtain **ribozymes** with the desired catalytic properties.

4/3,AB/22 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12599624 BIOSIS NO.: 200000353126
In vitro selection of self-cleaving **ribozymes** and deoxyribozymes.
BOOK TITLE: Intracellular **ribozyme** applications: Principles and protocols
AUTHOR: **Breaker Ronald R**(a
BOOK AUTHOR/EDITOR: Rossi John J; Couture Larry A: Authors
AUTHOR ADDRESS: (a)Department of Molecular Cellular and Developmental Biology, Yale University, New Haven, CT, 06520-8103**USA
JOURNAL: Intracellular ribozyme applications: Principles and protocolspl-19 1999
MEDIUM: print
BOOK PUBLISHER: Horizon Scientific Press, P. O. Box 1, Wymondham, Norfolk, NR18 0EH, UK
ISBN: 1-898486-17-4 (hardback)
DOCUMENT TYPE: Book
RECORD TYPE: Citation
LANGUAGE: English
SUMMARY LANGUAGE: English
1999

4/3,AB/23 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12449182 BIOSIS NO. 0000202684
Capping DNA with DNA.
AUTHOR: Li Yingfu; Liu Yong; **Breaker Ronald R**(a
AUTHOR ADDRESS: (a)Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, 06520-8103**USA
JOURNAL: Biochemistry 39 (11):p3106-3114 March 21, 2000
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: Twelve classes of deoxyribozymes that promote an ATP-dependent "self-capping" reaction were isolated by in vitro selection from a random-sequence pool of DNA. Each deoxyribozyme catalyzes the transfer of the AMP moiety of ATP to its 5'-terminal phosphate group, thereby forming a 5', 5'-pyrophosphate linkage. An identical DNA adenylate structure is generated by the T4 DNA ligase during enzymatic DNA ligation. A 41-nucleotide class 1 deoxyribozyme requires Cu2+ as a cofactor and adopts a structure that recognizes both the adenine and triphosphate moieties of ATP or dATP. The catalytic efficiency for this DNA, measured at 104 M-1cntdotmin-1 using either ATP or dATP as substrate, is similar to other catalytic nucleic acids that use small substrates. Chemical probing and site-directed mutagenesis implicate the formation of guanine quartets as critical components of the active structure. The observation of ATP-dependent "self-charging" by DNA suggests that DNA could be made to perform the reactions typically associated with DNA cloning, but without the assistance of protein enzymes.

2000

4/3,AB/24 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12437049 BIOSIS NO.: 200000190551
Engineering RNA and DNA biocatalysts.
AUTHOR: **Breaker Ronald R**(a
AUTHOR ADDRESS: (a)Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, 06520**USA
JOURNAL: Abstracts of Papers American Chemical Society 219 (1-2):pBIOL 92 2000
CONFERENCE/MEETING: 219th Meeting of the American Chemical Society. San Francisco, California, USA March 26-30, 2000
SPONSOR: American Chemical Society
ISSN: 0065-7727
RECORD TYPE: Citation
LANGUAGE: English
SUMMARY LANGUAGE: English
2000

4/3,AB/25 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12043286 BIOSIS NO.: 199900323805
Ligand-specific molecular switches composed of RNA.
AUTHOR: Soukup Garrett A(a); Tang Jin(a); Koizumi Makoto(a); **Breaker Ronald R**(a
AUTHOR ADDRESS: (a)Dept. of Biology, Yale University, New Haven, CT**USA
JOURNAL: FASEB Journal 13 (7):pA1387 April 23, 1999
CONFERENCE/MEETING: Annual Meeting of the American Societies for

[redacted]

Experimental Biology on Biochemistry and Molecular Biology 99 San Francisco, California, USA May 16-20, 1999
SPONSOR: American Societies for Experimental Biology
ISSN: 0892-6638
RECORD TYPE: Citation
LANGUAGE: English
1999

4/3,AB/26 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2001 BIOSIS. All rts. reserv.

09749086 BIOSIS NO.: 199598204004
In vitro evolution of **ribozymes**.
AUTHOR: Joyce Gerald F(a); Banerji Amrita; **Breaker Ronald R**; Bruick Richard; Dai Xiaochang; De Mesmaeker Alain; Raillard Sun-Ai; Tsang Joyce
AUTHOR ADDRESS: (a)Dep. Chem., Scripps Res. Inst., La Jolla, CA 92037**USA
JOURNAL: Journal of Cellular Biochemistry Supplement 0 (19A):p207 1995
CONFERENCE/MEETING: Keystone Symposium on Ribozymes: Basic Science and Therapeutic Applications Breckenridge, Colorado, USA January 15-21, 1995
ISSN: 0733-1959
RECORD TYPE: Citation
LANGUAGE: English
1995
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7/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11256939 21241686 PMID: 11345431
Generating new ligand-binding RNAs by affinity maturation and

[redacted]

disintegration of allosteric ribozymes.
Soukup GA; DeRose EC; Koizumi M; Breaker RR
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520-8103, USA.
RNA (United States) Apr 2001, 7 (4) p524-36, ISSN 1355-8382
Journal Code: CHB
Contract/Grant No.: GM559343, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: In Process
Allosteric ribozymes are engineered RNAs that operate as molecular switches whose rates of catalytic activity are modulated by the binding of specific effector molecules. New RNA molecular switches can be created by using "allosteric selection," a molecular engineering process that combines modular rational design and in vitro evolution strategies. In this report, we describe the characterization of 3',5'-cyclic nucleotide monophosphate (cNMP)-dependent hammerhead ribozymes that were created using allosteric selection (Koizumi et al., Nat Struct Biol, 1999, 6:1062-1071). Artificial phylogeny data generated by random mutagenesis and reselection of existing cGMP-, cCMP-, and cAMP-dependent ribozymes indicate that each is comprised of distinct effector-binding and catalytic domains. In addition, patterns of nucleotide covariation and direct mutational analysis both support distinct secondary-structure organizations for the effector-binding domains. Guided by these structural models, we were able to disintegrate each allosteric ribozyme into separate ligand-binding and catalytic modules. Examinations of the independent effector-binding domains reveal that each retains its corresponding cNMP-binding function. These results validate the use of allosteric selection and modular engineering as a means of simultaneously generating new nucleic acid structures that selectively bind ligands. Furthermore, we demonstrate that the binding affinity of an allosteric ribozyme can be improved through random mutagenesis and allosteric selection under conditions that favor tighter binding. This "affinity maturation" effect is expected to be a valuable attribute of allosteric selection as future endeavors seek to apply engineered allosteric ribozymes as biosensor components and as controllable genetic switches.

7/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11243658 21169368 PMID: 11266567
Cooperative binding of effectors by an allosteric ribozyme.
Jose AM; **Soukup GA**; Breaker RR
Department of Molecular, Cellular and Developmental Biology, KBT 452, Yale University, PO Box 208103, New Haven, CT 06520-8103, USA.
Nucleic acids research (England) Apr 1 2001, 29 (7) p1631-7, ISSN 1362-4962 Journal Code: DF1
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
An allosteric ribozyme that requires two different effectors to induce catalysis was created using modular rational design. This ribozyme construct comprises five conjoined RNA modules that operate in concert as an obligate FMN- and theophylline-dependent molecular switch. When both effectors are present, this 'binary' RNA switch self-cleaves with a rate enhancement of approximately 300-fold over the rate observed in the absence of effectors. Kinetic and structural studies implicate a switching mechanism wherein FMN binding induces formation of the active ribozyme conformation. However, the binding site for FMN is rendered inactive unless theophylline first binds to its corresponding site and reorganizes the RNA structure. This example of cooperative binding between allosteric effectors reveals a level of structural and functional complexity for RNA that is similar to that observed with allosteric proteins.

7/3,AB/3 (Item 3 [redacted]m file: 155)
DIALOG(R)File 155:MEDLINE(R)

11040129 21060020 PMID: 10542100
Allosteric selection of ribozymes that respond to the second messengers cGMP and cAMP.
Koizumi M; **Soukup GA**; Kerr JN; Breaker RR
Department of Molecular, Cellular and Developmental Biology, Yale University, P.O. Box 208103, New Haven, Connecticut 06520-8103, USA.
Nature structural biology (United States) Nov 1999, 6 (11) p1062-71, ISSN 1072-8368 Journal Code: B98
Languages: ENGLISH
Document type: Journal Article
Record type: In Process
RNA transcripts containing the hammerhead ribozyme have been engineered to self-destruct in the presence of specific nucleoside 3',5'-cyclic monophosphate compounds. These RNA molecular switches were created by a new combinatorial strategy termed 'allosteric selection,' which favors the emergence of ribozymes that rapidly self-cleave only when incubated with their corresponding effector compounds. Representative RNAs exhibit 5,000-fold activation upon cGMP or cAMP addition, display precise molecular recognition characteristics, and operate with catalytic rates that match those exhibited by unaltered ribozymes. These findings demonstrate that a vast number of ligand-responsive ribozymes with dynamic structural characteristics can be generated in a massively parallel fashion. Moreover, optimized allosteric ribozymes could serve as highly selective sensors of chemical agents or as unique genetic control elements for the programmed destruction of cellular RNAs.

7/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10673809 20311456 PMID: 10851196
Allosteric nucleic acid catalysts.
Soukup GA; Breaker RR
Department of Molecular, Cellular and Development Biology, Yale University, New Haven, Connecticut 06520-8103, USA.
Current opinion in structural biology (ENGLAND) Jun 2000, 10 (3) p318-25, ISSN 0959-440X Journal Code: B9V
Contract/Grant No.: GM559343, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Endowing nucleic acid catalysts with allosteric properties provides new prospects for RNA and DNA as controllable therapeutic agents or as sensors of their cognate effector compounds. The ability to engineer RNA catalysts that are allosterically regulated by effector binding has been propelled by the union of modular rational design principles and powerful combinatorial strategies.

7/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10628991 20241398 PMID: 10780486
Allosteric ribozymes sensitive to the second messengers cAMP and cGMP.
Koizumi M; Kerr JN; **Soukup GA**; Breaker RR
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA.
Nucleic acids symposium series (ENGLAND) 1999, (42) p275-6, ISSN 0261-3166 Journal Code: O8N
Languages: ENGLISH
Document type: Journal Article

[redacted]

Record type: Completed
We have engineered allosteric ribozymes by combining modular rational design with combinatorial strategies. This new procedure was used to create allosteric ribozymes that are activated by specific nucleoside 3',5'-cyclic monophosphates (cNMPs). A random-sequence domain was attached to stem II of hammerhead ribozymes via a communication module that serves as an interface between ribozyme and the effector binding site. Subjecting this initial random pool to in vitro selection methods produced populations that respond, or cleave, only in the presence of specific effector molecules. From generation 18, 20 and 23, cGMP, cCMP and cAMP-specific responsive ribozymes, respectively, were isolated and characterized. These methods show great promise for engineering allosteric ribozymes and for creating new ligand-specific aptamers.

7/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10605427 20251042 PMID: 10788325
Altering molecular recognition of RNA aptamers by allosteric selection.
Soukup GA; Emilsson GA; Breaker RR
Department of Molecular Cellular and Developmental Biology, Yale University, New Haven, CT 06520-8103, USA.
Journal of molecular biology (ENGLAND) May 12 2000, 298 (4) p623-32, ISSN 0022-2836 Journal Code: J6V
Contract/Grant No.: GM559343, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
In a continuing effort to explore structural and functional dynamics in RNA catalysis, we have created a series of allosteric hammerhead ribozymes that are activated by theophylline. Representative ribozymes exhibit greater than 3000-fold activation upon effector-binding and cleave with maximum rate constants that are equivalent to the unmodified hammerhead ribozyme. In addition, we have evolved a variant allosteric ribozyme that exhibits an effector specificity change from theophylline to 3-methylxanthine. Molecular discrimination between the two effectors appears to be mediated by subtle conformational differences that originate from displacement of the phosphodiester backbone near the effector binding pocket. These findings reveal the importance of abstruse aspects of molecular recognition by nucleic acids that are likely to be unapproachable by current methods of rational design. Copyright 2000 Academic Press.

7/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10422985 20025500 PMID: 10557159
Nucleic acid molecular switches.
Soukup GA; Breaker RR
Department of Molecular, Cellular and Developmental Biology, Yale University, PO Box 208103, New Haven, CT 06520-8103, USA. garrett.soukup@yale.edu
Trends in biotechnology (ENGLAND) Dec 1999, 17 (12) p469-76, ISSN 0167-7799 Journal Code: ALJ
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed
Natural and artificial ribozymes can catalyse a diverse range of chemical reactions. Through recent efforts in enzyme engineering, it has become possible to tailor the activity of ribozymes to respond allosterically to specific effector compounds. These allosteric ribozymes function as effector-dependent molecular switches that could find application as novel genetic-control elements, biosensor components or precision switches for use in nanotechnology.

[redacted]

7/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10392264 20037793 PMID: 10573122
Relationship between internucleotide linkage geometry and the stability of RNA.
Soukup GA; Breaker RR
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520-8103, USA.
RNA (UNITED STATES) Oct 1999, 5 (10) p1308-25, ISSN 1355-8382 Journal Code: CHB
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The inherent chemical instability of RNA under physiological conditions is primarily due to the spontaneous cleavage of phosphodiester linkages via intramolecular transesterification reactions. Although the protonation state of the nucleophilic 2'-hydroxyl group is a critical determinant of the rate of RNA cleavage, the precise geometry of the chemical groups that comprise each internucleotide linkage also has a significant impact on cleavage activity. Specifically, transesterification is expected to be proportional to the relative in-line character of the linkage. We have examined the rates of spontaneous cleavage of various RNAs for which the secondary and tertiary structures have previously been modeled using either NMR or X-ray crystallographic data. Rate constants determined for the spontaneous cleavage of different RNA linkages vary by almost 10,000-fold, most likely reflecting the contribution that secondary and tertiary structures make towards the overall chemical stability of RNA. Moreover, a correlation is observed between RNA cleavage rate and the relative in-line fitness of each internucleotide linkage. One linkage located within an ATP-binding RNA aptamer is predicted to adopt most closely the ideal conformation for in-line attack. This linkage has a rate constant for transesterification that is approximately 12-fold greater than is observed for an unconstrained linkage and was found to be the most labile among a total of 136 different sites examined. The implications of this relationship for the chemical stability of RNA and for the mechanisms of nucleases and ribozymes are discussed.

7/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10345594 99354412 PMID: 10425680
Design of allosteric hammerhead ribozymes activated by ligand-induced structure stabilization.
Soukup GA; Breaker RR
Department of Molecular, Yale University, New Haven, Connecticut 06520-8103, USA.
Structure with Folding & design (ENGLAND) Jul 15 1999, 7 (7) p783-91 , ISSN 0969-2126 Journal Code: DEB
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
BACKGROUND: Ribozymes can function as allosteric enzymes that undergo a conformational change upon ligand binding to a site other than the active site. Although allosteric ribozymes are not known to exist in nature, nucleic acids appear to be well suited to display such advanced forms of kinetic control. Current research explores the mechanisms of allosteric ribozymes as well as the strategies and methods that can be used to create new controllable enzymes. RESULTS: In this study, we exploit the modular nature of certain functional RNAs to engineer allosteric ribozymes that are activated by flavin mononucleotide (FMN) or theophylline. By joining an FMN- or theophylline-binding domain to a hammerhead ribozyme by different

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stem II elements, we have identified a minimal connective bridge comprised of a G.U wobble pair that is responsive to ligand binding. Binding of FMN or theophylline to its allosteric site induces a conformational change in the RNA that stabilizes the wobble pair and ultimately favors the active form of the catalytic core. These ligand-sensitive ribozymes exhibit rate enhancements of more than 100-fold in the presence of FMN and of approximately 40-fold in the presence of theophylline. CONCLUSIONS: An adaptive strategy for modular rational design has proven to be an effective approach to the engineering of novel allosteric ribozymes. This strategy was used to create allosteric ribozymes that function by a mechanism involving ligand-induced structure stabilization. Conceivably, similar engineering strategies and allosteric mechanisms could be used to create a variety of novel allosteric ribozymes that function with other effector molecules.

7/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10112907 99199227 PMID: 10097080
Engineering precision RNA molecular switches.
Soukup GA; Breaker RR
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520-8103, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 30 1999, 96 (7) p3584-9, ISSN 0027-8424 Journal Code: PV3
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Ligand-specific molecular switches composed of RNA were created by coupling preexisting catalytic and receptor domains via structural bridges. Binding of ligand to the receptor triggers a conformational change within the bridge, and this structural reorganization dictates the activity of the adjoining ribozyme. The modular nature of these tripartite constructs makes possible the rapid construction of precision RNA molecular switches that trigger only in the presence of their corresponding ligand. By using similar enzyme engineering strategies, new RNA switches can be made to operate as designer molecular sensors or as a new class of genetic control elements.

7/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09797842 98256444 PMID: 9592159
Selection and characterization of RNAs that relieve transcriptional interference in Escherichia coli.
Soukup GA; Maher JJ
Department of Biochemistry and Molecular Biology, Mayo Foundation, Guggenheim 16, 200 First Street SW, Rochester, MN 55905, USA.
Nucleic acids research (ENGLAND) Jun 1 1998, 26 (11) p2715-22, ISSN 0305-1048 Journal Code: O8L
Contract/Grant No.: GM47814, GM, NIGMS; GM54411, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Oligonucleotide-directed triple helix formation offers a method for duplex DNA recognition, and has been proposed as an approach to the rational design of gene-specific repressors. Indeed, certain RNA and DNA oligonucleotides have previously been shown to bind duplex DNA and repress in vitro transcription by occluding the binding of transcription factors or RNA polymerase at target genes. While similar oligonucleotides have reportedly caused repression of target genes in cultured cells, physical evidence of triple helix formation in vivo is generally lacking. In the

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present study we wished to determine whether RNA transcripts could repress the activity of an Escherichia coli promoter in vivo by binding to the duplex promoter DNA. An in vivo genetic selection previously developed to identify DNA binding proteins was modified for this purpose. Using expression libraries encoding RNAs predisposed to forming triple helices with a DNA target site, we have selected RNA transcripts that confer survival to E.coli by disrupting transcriptional interference. Surprisingly, genetic and biochemical evidence shows that these RNAs do not form triple helices at the target promoter in vivo , despite the fact that they contain sequences capable of forming triple helices at the duplex DNA target in vitro . Rather, the selected RNAs appear to disrupt transcriptional interference via an antisense mechanism.

7/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08919500 96228248 PMID: 8656424
Selection of RNAs that bind to duplex DNA at neutral pH.
Soukup GA; Ellington AD; Maher LJ
Department of Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN 55905, USA.
Journal of molecular biology (ENGLAND) Jun 7 1996, 259 (2) p216-28, ISSN 0022-2836 Journal Code: J6V
Contract/Grant No.: AI36083, AI, NIAID; GM47814, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
RNA that are capable of binding duplex DNA in a site-specific manner have potential applications in gene therapy strategies. Such RNAs might be targeted to DNA sequences in a gene promoter and prevent initiation of transcription by occluding transcription factors and/or RNA polymerases. RNA oligonucleotides that bind homopurine/homopyrimidine DNA sequences by forming triple-helical complexes involving T.A.T and C+.G.C base-triplets can be rationally designed. However, the formation of such pyrimidine motif triple helices typically requires mildly acidic conditions. In addition, the proper oligonucleotide sequence must be optimally presented within a longer RNA transcript if it is to be synthesized in vivo. To address these issues, RNAs were selected from pools of random sequences for binding to a homopurine/homopyrimidine DNA sequence. RNAs selected for binding the duplex DNA target between pH 6.5 and pH 7.4 were characterized by sequence analysis and binding studies. All RNAs isolated by selection and amplification were found to contain a pyrimidine recognition sequence for binding the duplex DNA target via conventional triple helix formation. The selected approximately 85 nt RNAs have dissociation constants that approach, but do not surpass, the binding affinity of a 21 nt RNA oligonucleotide that binds the DNA target sequence by forming a canonical triple helix. The presence of a pyrimidine recognition sequence within a longer RNA transcript is not sufficient for high affinity. Experimental data and secondary structure predictions suggest that the context of the pyrimidine recognition sequence within selected RNAs is a very important determinant of DNA binding affinity. These studies provide insight into the development of RNA transcripts that may function as gene-specific repressors by forming triple helices with DNA in vivo.

7/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08490367 95226484 PMID: 7711100
Preparation of oligonucleotide-biotin conjugates with cleavable linkers.
Soukup GA; Cerny RL; Maher LJ
Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha 68198-6805, USA.
Bioconjugate chemistry (UNITED STATES) Jan-Feb 1995, 6 (1) p135-8,

[redacted]

ISSN 1043-1802 Journal Code: A1T
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

A procedure is presented for preparing an oligonucleotide-biotin conjugate that is chemically cleavable through the reduction of a disulfide bond within the linker. Conjugation involves reaction of a primary amine with an N-hydroxysulfosuccinimide ester linked to biotin. The oligonucleotide can be liberated from streptavidin agarose containing immobilized conjugate under mild conditions (neutral pH, 50 mM dithiothreitol). This cleavable conjugate is useful for affinity purification applications.

12043286 BIOSIS NO.: 199900323805
Ligand-specific molecular switches composed of RNA.
AUTHOR: **Soukup Garrett A**(a); Tang Jin(a); Koizumi Makoto(a); Breaker Ronald R(a
AUTHOR ADDRESS: (a)Dept. of Biology, Yale University, New Haven, CT**USA
JOURNAL: FASEB Journal 13 (7):pA1337 April 23, 1999
CONFERENCE/MEETING: Annual Meeting of the American Societies for Experimental Biology on Biochemistry and Molecular Biology 99 San Francisco, California, USA May 16-20, 1999
SPONSOR: American Societies for Experimental Biology
ISSN: 0892-6638
RECORD TYPE: Citation
LANGUAGE: English

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         1260571  IN(W)VITRO
          111468  CATALYTIC
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      S6      16  RD (unique items)
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 6/3,AB/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11360739   21441323   PMID: 11557347
  Characterization of a DNA-cleaving deoxyribozyme.
  Carmi N; Breaker R R
  Department of Molecular, Cellular and Developmental Biology, Yale
University, New Haven, CT 06520-8103, USA.
  Bioorganic & medicinal chemistry (England) Oct 2001, 9 (10)
 p2589-600, ISSN 0968-0896 Journal Code: 9413298
  Document type: Journal Article
  Languages: ENGLISH
  Main Citation Owner: NLM
  Record type: Completed
  A copper-dependent self-cleaving DNA that was isolated by in
vitro selection has been minimized to its smallest active domain
using both in vitro selection and rational design methods. The
minimized 46-nucleotide deoxyribozyme forms duplex and triplex
substructures that flank a highly conserved catalytic core. This
self-cleaving construct can be converted into a bimolecular complex that
comprises separate substrate and enzyme domains. Substrate cleavage is
directed at one of two adjacent nucleotides and proceeds via an oxidative
cleavage mechanism that is unique to the position cleaved. The structural,
kinetic and mechanistic characteristics of this DNA-cleaving deoxyribozyme
are reported.


 6/3,AB/2     (Item 2 from file: 155)
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DIALOG(R)File 155:MEDLINE(R)

11214278 21241686 PMID: 11345431
Generating new ligand-binding RNAs by affinity maturation and disintegration of allosteric ribozymes.
Soukup G A; DeRose E C; Koizumi M; **Breaker R R**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520-8103, USA.
RNA (New York, N.Y.) (United States) Apr 2001, 7 (4) p524-36, ISSN 1355-8382 Journal Code: 9509184
Contract/Grant No.: GM559343; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Allosteric ribozymes are engineered RNAs that operate as molecular switches whose rates of **catalytic** activity are modulated by the binding of specific effector molecules. New RNA molecular switches can be created by using "allosteric selection," a molecular engineering process that combines modular rational design and **in vitro** evolution strategies. In this report, we describe the characterization of 3',5'-cyclic nucleotide monophosphate (cNMP)-dependent hammerhead ribozymes that were created using allosteric selection (Koizumi et al., Nat Struct Biol, 1999, 6:1062-1071). Artificial phylogeny data generated by random mutagenesis and reselection of existing cGMP-, cCMP-, and cAMP-dependent ribozymes indicate that each is comprised of distinct effector-binding and **catalytic** domains. In addition, patterns of nucleotide covariation and direct mutational analysis both support distinct secondary-structure organizations for the effector-binding domains. Guided by these structural models, we were able to disintegrate each allosteric ribozyme into separate ligand-binding and **catalytic** modules. Examinations of the independent effector-binding domains reveal that each retains its corresponding cNMP-binding function. These results validate the use of allosteric selection and modular engineering as a means of simultaneously generating new nucleic acid structures that selectively bind ligands. Furthermore, we demonstrate that the binding affinity of an allosteric ribozyme can be improved through random mutagenesis and allosteric selection under conditions that favor tighter binding. This "affinity maturation" effect is expected to be a valuable attribute of allosteric selection as future endeavors seek to apply engineered allosteric ribozymes as biosensor components and as controllable genetic switches.

6/3,AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11098469 21110474 PMID: 11181037
In vitro selection of kinase and ligase deoxyribozymes.
Li Y; **Breaker R R**
Department of Molecular, Cellular, and Developmental Biology, Yale University, 219 Prospect Street, New Haven, Connecticut 06520-8103, USA.
Methods (San Diego, Calif.) (United States) Feb 2001, 23 (2) p179-90, ISSN 1046-2023 Journal Code: 9426302
Contract/Grant No.: GM57500; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Exploration of the limits of biocatalysis has led to the discovery that DNA has significant potential for enzymatic function. This makes possible the construction of DNA enzymes or "deoxyribozymes" for catalyzing various chemical reactions that could be used to address fundamental questions in biocatalysis or that could find unique applications in biotechnology. Of significant interest are self-modification reactions, given the fundamental

role that DNA serves in modern living systems. Recently, **in vitro** selection strategies have been used to isolate prototypical ATP-dependent deoxyribozymes from random-sequence populations of DNA that catalyze DNA phosphorylation and others that catalyze DNA adenylation. In nature, protein enzymes such as T4 DNA kinase and T4 DNA ligase catalyze identical chemical reactions. These findings suggest that DNA constructs could be engineered to efficiently catalyze other self-modifying reactions, including ATP-dependent DNA ligation. This article provides a detailed overview of the methods used to isolate deoxyribozymes that promote ATP-dependent DNA ligation. Copyright 2001 Academic Press.

6/3,AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10750168 20283903 PMID: 10823936
Structural diversity of self-cleaving ribozymes.
Tang J; **Breaker R R**
Department of Molecular, Cellular and Developmental Biology, Yale University, P.O. Box 208103, New Haven, CT 06520-8103, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) May 23 2000, 97 (11) p5784-9, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In vitro selection was used to isolate Mg(2+)-dependent self-cleaving ribozymes from random sequence. Characterization of representative clones revealed the emergence of at least 12 classes of ribozymes that adopt distinct secondary structure motifs. Only one class corresponds to a previously known structural motif, that of the naturally occurring hammerhead ribozyme. Each ribozyme promotes self-cleavage via an internal phosphoester transfer reaction involving the adjacent 2'-hydroxyl group with a chemical rate enhancement of between 10(3)- and 10(6)-fold greater than the corresponding uncatalyzed rate. These findings indicate that RNA can form a multitude of secondary and tertiary structures that promote cleavage by internal phosphoester transfer. Upon further **in vitro** selection, a class I ribozyme that adopts an "X motif" structure dominates over all other ribozymes in the population. Thus, self-cleaving RNAs isolated by **in vitro** selection from random-sequence populations can rival the **catalytic** efficiency of natural ribozymes.

6/3,AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10705011 20241398 PMID: 10780486
Allosteric ribozymes sensitive to the second messengers cAMP and cGMP.
Koizumi M; Kerr J N; Soukup G A; **Breaker R R**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA.
Nucleic acids symposium series (ENGLAND) 1999, (42) p275-6, ISSN 0261-3166 Journal Code: 8007206
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have engineered allosteric ribozymes by combining modular rational design with combinatorial strategies. This new procedure was used to create allosteric ribozymes that are activated by specific nucleoside 3',5'-cyclic monophosphates (cNMPs). A random-sequence domain was attached to stem II of hammerhead ribozymes via a communication module that serves as an interface

between ribozyme and the effector binding site. Subjecting this initial random pool to **in vitro** selection methods produced populations that respond, or cleave, only in the presence of specific effector molecules. From generation 18, 20 and 23, cGMP, cCMP and cAMP-specific responsive ribozymes, respectively, were isolated and characterized. These methods show great promise for engineering allosteric ribozymes and for creating new ligand-specific aptamers.

6/3,AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10704990 20241379 PMID: 10780467
In vitro selection of deoxyribozymes with DNA capping activity.
Li Y; Liu Y; **Breaker R R**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA.
Nucleic acids symposium series (ENGLAND) 1999, (42) p237-8, ISSN 0261-3166 Journal Code: 8007206
Contract/Grant No.: GM57500; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
In vitro selection was used to isolate a series of deoxyribozymes from a pool of random-sequence DNAs that catalyze an ATP-dependent self-capping reaction. Each deoxyribozyme catalyzes the transfer of the nucleoside and alpha-phosphate moieties of ATP to the phosphate group located at its 5' terminus, thereby creating a 5',5'-pyrophosphate cap. This same pyrophosphate cap structure is formed by T4 DNA ligase during the classical process of DNA ligation. These DNA capping enzymes representative of a collection of self-processing deoxyribozymes that can be used for the directed modification of DNA.

6/3,AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10640497 20181684 PMID: 10715132
Capping DNA with DNA.
Li Y; Liu Y; **Breaker R R**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520-8103, USA.
Biochemistry (UNITED STATES) Mar 21 2000, 39 (11) p3106-14, ISSN 0006-2960 Journal Code: 0370623
Contract/Grant No.: GM57500; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Twelve classes of deoxyribozymes that promote an ATP-dependent "self-capping" reaction were isolated by **in vitro** selection from a random-sequence pool of DNA. Each deoxyribozyme catalyzes the transfer of the AMP moiety of ATP to its 5'-terminal phosphate group, thereby forming a 5',5'-pyrophosphate linkage. An identical DNA adenylate structure is generated by the T4 DNA ligase during enzymatic DNA ligation. A 41-nucleotide class 1 deoxyribozyme requires Cu(2+) as a cofactor and adopts a structure that recognizes both the adenine and triphosphate moieties of ATP or dATP. The **catalytic** efficiency for this DNA, measured at 10(4) M(-1) x min(-1) using either ATP or dATP as substrate, is similar to other **catalytic** nucleic acids that use small substrates. Chemical probing and site-directed mutagenesis implicate the formation of guanine quartets as critical components of the active structure. The

observation of ATP-dependent "self-charging" by DNA suggests that DNA could be made to perform the reactions typically associated with DNA cloning, but without the assistance of protein enzymes.

6/3,AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10405030 99383018 PMID: 10453642
In vitro selection of nucleic acid enzymes.
Kurz M; **Breaker R R**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520-8103, USA.
Current topics in microbiology and immunology (GERMANY) 1999, 243 p137-58, ISSN 0070-217X Journal Code: 0110513
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

6/3,AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10308950 99290830 PMID: 10361095
Deoxyribozymes: new players in the ancient game of biocatalysis.
Li Y; **Breaker R R**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520-8103, USA.
Current opinion in structural biology (ENGLAND) Jun 1999, 9 (3) p315-23, ISSN 0959-440X Journal Code: 9107784
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The repetitive and extraordinarily stable polynucleotide chains of DNA serve as an ideal storage system for genetic information. Although it is best known for its helical structure and relatively inert character, **in vitro** selection can be used to compel DNA to perform a surprising variety of chemical reactions. These artificial DNA enzymes or 'deoxyribozymes' generate large chemical rate enhancements and demonstrate precise substrate recognition, much like their protein and RNA counterparts. Recent studies with these prototypic deoxyribozymes indicate that DNA has a substantial untapped potential for intricate structure formation that could be exploited in novel chemical and biological catalysis.

6/3,AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10204312 99178960 PMID: 10077582
Phosphorylating DNA with DNA.
Li Y; **Breaker R R**
Department of Molecular, Cellular, and Developmental Biology, Yale University, P.O. Box 208103, New Haven, CT 06520-8103, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Mar 16 1999, 96 (6) p2746-51, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: GM57500; GM; NIGMS
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Nearly 50 individual DNAs with polynucleotide kinase-like activity were isolated from a random-sequence pool by using **in vitro** selection. Each self-phosphorylating deoxyribozyme makes use of one or more of the eight standard NTPs or dNTPs as a source of activated phosphate. Although most prototypic deoxyribozymes poorly differentiate between the ribose and deoxyribose moieties, further optimization by **in vitro** selection produced variants that display up to 100-fold discrimination between related NTP and dNTP substrates. An optimized ATP-dependent deoxyribozyme uses ATP >40,000-fold more efficiently than CTP, GTP, or UTP. This enzyme operates with a rate enhancement of nearly one billion-fold over the uncatalyzed rate of ATP hydrolysis. A bimolecular version of the ATP-dependent deoxyribozyme was further engineered to phosphorylate specific target DNAs with multiple turnover. The substrate-recognition patterns and rate enhancements intrinsic to these DNAs are characteristic of naturally occurring RNA and protein enzymes, supporting the hypothesis that DNA has sufficient **catalytic** potential to function as an enzyme in biological systems.

6/3,AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09833824 98263302 PMID: 9600911
An amino acid as a cofactor for a **catalytic** polynucleotide.
Roth A; **Breaker R R**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520-8103, USA.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) May 26 1998, 95 (11) p6027-31, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Natural ribozymes require metal ion cofactors that aid both in structural folding and in chemical catalysis. In contrast, many protein enzymes produce dramatic rate enhancements using only the chemical groups that are supplied by their constituent amino acids. This fact is widely viewed as the most important feature that makes protein a superior polymer for the construction of biological catalysts. Herein we report the **in vitro** selection of a **catalytic** DNA that uses histidine as an active component for an RNA cleavage reaction. An optimized deoxyribozyme from this selection requires L-histidine or a closely related analog to catalyze RNA phosphoester cleavage, producing a rate enhancement of approximately 1-million-fold over the rate of substrate cleavage in the absence of enzyme. Kinetic analysis indicates that a DNA-histidine complex may perform a reaction that is analogous to the first step of the proposed **catalytic** mechanism of RNase A, in which the imidazole group of histidine serves as a general base catalyst. Similarly, ribozymes of the "RNA world" may have used amino acids and other small organic cofactors to expand their otherwise limited **catalytic** potential.

6/3,AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09611501 98044719 PMID: 9383471
A DNA enzyme with Mg(2+)-dependent RNA phosphoesterase activity.
Breaker R R; Joyce G F Joyce G F Scripps Res Inst, La Jolla, CA
Department of Chemistry, Scripps Research Institute, La Jolla, CA 92037, USA.
Chemistry & biology (ENGLAND) Oct 1995, 2 (10) p655-60, ISSN 1074-5521 Journal Code: 9500160
Document type: Journal Article

[redacted]

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Previously we demonstrated that DNA can act as an enzyme in the Pb(2+)-dependent cleavage of an RNA phosphoester. This is a facile reaction, with an uncatalyzed rate for a typical RNA phosphoester of approximately 10(-4) min-1 in the presence of 1 mM Pb(OAc)2 at pH 7.0 and 23 degrees C. The Mg(2+)-dependent reaction is more difficult, with an uncatalyzed rate of approximately 10(-7) min-1 under comparable conditions. Mg(2+)-dependent cleavage has special relevance to biology because it is compatible with intracellular conditions. Using **in vitro** selection, we sought to develop a family of phosphoester-cleaving DNA enzymes that operate in the presence of various divalent metals, focusing particularly on the Mg(2+)-dependent reaction. RESULTS: We generated a population of > 10(13) DNAs containing 40 random nucleotides and carried out repeated rounds of selective amplification, enriching for molecules that cleave a target RNA phosphoester in the presence of 1 mM Mg2+, Mn2+, Zn2+ or Pb2+. Examination of individual clones from the Mg2+ lineage after the sixth round revealed a **catalytic** motif comprised of a three-stem junction. This motif was partially randomized and subjected to seven additional rounds of selective amplification, yielding catalysts with a rate of 0.01 min-1. The optimized DNA catalyst was divided into separate substrate and enzyme domains and shown to have a similar level of activity under multiple turnover conditions. CONCLUSIONS: We have generated a Mg(2+)-dependent DNA enzyme that cleaves a target RNA phosphoester with a **catalytic** rate approximately 10(5)-fold greater than that of the uncatalyzed reaction. This activity is compatible with intracellular conditions, raising the possibility that DNA enzymes might be made to operate in vivo.

6/3,AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09611488 98044642 PMID: 9383394
A DNA enzyme that cleaves RNA.
Breaker R R; Joyce G F Hoyce G F Scripps Res Inst, La Jolla, CA
Department of Chemistry, Scripps Research Institute, La Jolla, CA 92037, USA.
Chemistry & biology (ENGLAND) Dec 1994, 1 (4) p223-9, ISSN 1074-5521 Journal Code: 9500160
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Several types of RNA enzymes (ribozymes) have been identified in biological systems and generated in the laboratory. Considering the variety of known RNA enzymes and the similarity of DNA and RNA, it is reasonable to imagine that DNA might be able to function as an enzyme as well. No such DNA enzyme has been found in nature, however. We set out to identify a metal-dependent DNA enzyme using **in vitro** selection methodology. RESULTS: Beginning with a population of 10(14) DNAs containing 50 random nucleotides, we carried out five successive rounds of selective amplification, enriching for individuals that best promote the Pb(2+)-dependent cleavage of a target ribonucleoside 3'-O-P bond embedded within an otherwise all-DNA sequence. By the fifth round, the population as a whole carried out this reaction at a rate of 0.2 min-1. Based on the sequence of 20 individuals isolated from this population, we designed a simplified version of the **catalytic** domain that operates in an intermolecular context with a turnover rate of 1 min-1. This rate is about 10(5)-fold increased compared to the uncatalyzed reaction. CONCLUSIONS: Using **in vitro** selection techniques, we obtained a DNA enzyme that catalyzes the Pb(2+)-dependent cleavage of an RNA phosphoester in a reaction that proceeds with rapid turnover. The **catalytic** rate

compares favorably to that of known RNA enzymes. We expect that other examples of DNA enzymes will soon be forthcoming.

6/3,AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09499771 97400133 PMID: 9257650
Examination of the **catalytic** fitness of the hammerhead ribozyme by **in vitro** selection.
Tang J; **Breaker R R**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut 06520-8103, USA.
RNA (New York, N.Y.) (UNITED STATES) Aug 1997, 3 (8) p914-25, ISSN 1355-8382 Journal Code: 9509184
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have designed a self-cleaving ribozyme construct that is rendered inactive during preparative **in vitro** transcription by allosteric interactions with ATP. This allosteric ribozyme was constructed by joining a hammerhead domain to an ATP-binding RNA aptamer, thereby creating a ribozyme whose **catalytic** rate can be controlled by ATP. Upon purification by PAGE, the engineered ribozyme undergoes rapid self-cleavage when incubated in the absence of ATP. This strategy of "allosteric delay" was used to prepare intact hammerhead ribozymes that would otherwise self-destruct during transcription. Using a similar strategy, we have prepared a combinatorial pool of RNA in order to assess the **catalytic** fitness of ribozymes that carry the natural consensus sequence for the hammerhead. Using **in vitro** selection, this comprehensive RNA pool was screened for sequence variants of the hammerhead ribozyme that also display **catalytic** activity. We find that sequences that comprise the core of naturally occurring hammerhead dominate the population of selected RNAs, indicating that the natural consensus sequence of this ribozyme is optimal for **catalytic** function.

6/3,AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09475035 97370078 PMID: 9224568
Rational design of allosteric ribozymes.
Tang J; **Breaker R R**
Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, 06520-8103, USA.
Chemistry & biology (ENGLAND) Jun 1997, 4 (6) p453-9, ISSN 1074-5521 Journal Code: 9500160
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Efficient operation of cellular processes relies on the strict control that each cell exerts over its metabolic pathways. Some protein enzymes are subject to allosteric regulation, in which binding sites located apart from the enzyme's active site can specifically recognize effector molecules and alter the **catalytic** rate of the enzyme via conformational changes. Although RNA also performs chemical reactions, no ribozymes are known to operate as true allosteric enzymes in biological systems. It has recently been established that small-molecule receptors can readily be made of RNA, as demonstrated by the **in vitro** selection of various RNA aptamers that can specifically bind corresponding ligand molecules. We set out to examine whether the **catalytic** activity of an existing ribozyme could be brought under the

control of an effector molecule by designing conjoined aptamer-ribozyme complexes. RESULTS: By joining an ATP-binding RNA to a self-cleaving ribozyme, we have created the first example of an allosteric ribozyme that has a **catalytic** rate that can be controlled by ATP. A 180-fold reduction in rate is observed upon addition of either adenosine or ATP, but no inhibition is detected in the presence of dATP or other nucleoside triphosphates. Mutations in the aptamer domain that are expected to eliminate ATP binding or that increase the distance between aptamer and ribozyme domains result in a loss of ATP-specific allosteric control. Using a similar design approach, allosteric hammerhead ribozymes that are activated in the presence of ATP were created and another ribozyme that can be controlled by theophylline was created. CONCLUSIONS: The **catalytic** features of these conjoined aptamer-ribozyme constructs demonstrate that **catalytic** RNAs can also be subject to allosteric regulation-a key feature of certain protein enzymes. Moreover, by using simple rational design strategies, it is now possible to engineer new **catalytic** polynucleotides which have rates that can be tightly and specifically controlled by small effector molecules.

6/3,AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09254627 97153183 PMID: 9000012
In vitro selection of self-cleaving DNAs.
Carmi N; Shultz L A; **Breaker R R**
Department of Biology, Yale University, New Haven, CT 06520-8103, USA.
Chemistry & biology (ENGLAND) Dec 1996, 3 (12) p1039-46, ISSN 1074-5521 Journal Code: 9500160
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Ribozymes catalyze an important set of chemical transformations in metabolism, and 'engineered' ribozymes have been made that catalyze a variety of additional reactions. The possibility that **catalytic** DNAs or 'deoxyribozymes' can be made has only recently been addressed. Specifically, it is unclear whether the absence of the 2' hydroxyl renders DNA incapable of exhibiting efficient enzyme-like activity, making it impossible to discover natural or create artificial DNA biocatalysts. RESULTS: We report the isolation by **in vitro** selection of two distinct classes of self-cleaving DNAs from a pool of random-sequence oligonucleotides. Individual catalysts from 'class I' require both Cu2+ and ascorbate to mediate oxidative self-cleavage. Individual catalysts from class II use Cu2+ as the sole cofactor. Further optimization of a class II individual by **in vitro** selection yielded new **catalytic** DNAs that facilitate Cu2+-dependent self-cleavage with rate enhancements exceeding 1 000 000-fold relative to the uncatalyzed rate of DNA cleavage. CONCLUSIONS: Despite the absence of 2' hydroxyls, single-stranded DNA can adopt structures that promote divalent-metal-dependent self-cleavage via an oxidative mechanism. These results suggest that an efficient DNA enzyme might be made to cleave DNA in a biological context.